

**Amendments to the Claims:**

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims:**

Claims 1-256 (canceled)

Claim 257 (new): A method for inducing an antigen-specific immune response in a subject comprising:

a) pretreating an area of the skin of the subject, wherein pretreating comprises applying means for enhancing penetration and/or barrier disruption of the skin; and

b) applying a formulation transcutaneously to the pretreated area to induce an antigen-specific immune response, wherein the formulation comprises:

- 1) an antigen in an amount effective to induce an antigen-specific immune response;
  - 2) an adjuvant present in an amount effective to enhance the immune response to the antigen; and,
  - 3) a pharmaceutically acceptable carrier;
- wherein pretreating enhances the immune response.

Claim 258 (new): The method of claim 257, wherein pretreating comprises applying to the skin a chemical means, a physical means, a mechanical means, a hydration means, or a combination thereof.

Claim 259 (new): The method of claim 257, wherein pretreating comprises applying a chemical to the skin.

Claim 260 (new): The method of claim 259, wherein the chemical is an acetone, a detergent, a depilatory agent, a keratinolytic formulation, or a combination thereof.

Claim 261 (new): The method of claim 257, wherein pretreating comprises applying a device.

Claim 262 (new): The method of claim 261, wherein the device is selected from the group consisting of a propellant device, a device comprising tines, a device comprising microneedles, a device comprising a tine disk, a tape stripping device, a gas powered gun, a swab, an emery board, an abrasive pad, an electroporation device, an ultrasound device, and an iontophoresis device.

Claim 263 (new): The method of claim 261, wherein a patch comprises the device.

Claim 264 (new): The method of claim 257, wherein the antigen is a nucleic acid, carbohydrate, a glycolipid, a glycoprotein, a lipid, a lipoprotein, phospholipid, a polypeptide, a protein, a fusion protein, or chemical conjugate of a combination thereof.

Claim 265 (new): The method of claim 257, wherein the antigen is derived from a pathogen.

Claim 266 (new): The method of claim 265, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

Claim 267 (new): The method of claim 266, wherein the virus is an influenza virus or a rabies virus.

Claim 268 (new): The method of claim 267, wherein the antigen is hemagglutinin A.

Claim 269 (new): The method of claim 266, wherein the bacterium is *E. coli* or *Bacillus anthracis*.

Claim 270 (new): The method of claim 269, wherein the antigen is *E. coli* heat-labile enterotoxin (LT).

Claim 271 (new): The method of claim 269, wherein the antigen is a nucleic acid encoding *E. coli* heat-labile enterotoxin (LT).

Claim 272 (new): The method of claim 257, wherein the antigen is a pathogen.

Claim 273 (new): The method of claim 272, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

Claim 274 (new): The method of claim 273, wherein the virus is a whole virus, a live virus, an attenuated live virus, an inactivated virus, a detergent treated virus, or a combination thereof.

Claim 275 (new): The method of claim 274, wherein the virus is an influenza virus or a rabies virus.

Claim 276 (new): The method of claim 275, wherein the influenza virus comprises hemagglutinin A.

Claim 277 (new): The method of claim 273, wherein the bacterium is *E. coli* or *Bacillus anthracis*.

Claim 278 (new): The method of claim 277, wherein the *E. coli* comprises *E. coli* heat-labile enterotoxin (LT).

Claim 279 (new): The method of claim 257, wherein the antigen is a multivalent antigen.

Claim 280 (new): The method of claim 257, wherein the adjuvant comprises a molecule

selected from the group consisting of a bacterial ADP-ribosylating exotoxin (bARE), a binding B subunit of a bARE, a toxoid of a bARE, a genetically altered bARE, and a genetically detoxified mutant of a bARE.

Claim 281 (new): The method of claim 280, wherein the antigen is an influenza antigen.

Claim 282 (new): The method of claim 257, wherein the adjuvant is a nucleic acid encoding a molecule selected from the group consisting of a bacterial ADP-ribosylating exotoxin (bARE), a binding B subunit of a bARE, a toxoid of a bARE, a genetically altered bARE, and a genetically detoxified mutant of a bARE.

Claim 283 (new): The method of claim 281, wherein the nucleic acid encodes *E. coli* heat labile enterotoxin (LT).

Claim 284 (new): The method of claim 257, wherein the antigen and the adjuvant are the same molecule.

Claim 285 (new): The method of claim 284, wherein the molecule is *E. coli* heat-labile enterotoxin (LT).

Claim 286 (new): The method of claim 284, wherein the molecule is hemagglutinin A.

Claim 287 (new): The method of 257, wherein the formulation is applied using a patch.

Claim 288 (new): The method of claim 257, wherein the adjuvant is selected from the group consisting of nucleic acid, bacterial exotoxin, cytokine, chemokine, lipopolysaccharide, a molecule containing unmethylated CpG motifs, a heat shock protein, a derivative of a heat shock protein, tumor necrosis factor, genetically detoxified toxin, and combinations thereof.

Claim 289 (new): The method of claim 257, wherein the adjuvant is provided as a

nucleic acid comprising a sequence encoding the adjuvant.

Claim 290 (new): The method of claim 257, wherein the antigen or adjuvant activates an antigen presenting cell.

Claim 291 (new): The method of claim 290, wherein the antigen presenting cell is a Langerhans cell or a dermal dendritic cell.

Claim 292 (new): The method of claim 257, wherein the antigen is a whole microorganism, a whole cell, or a virion.

Claim 293 (new): A method for inducing an antigen-specific immune response in a subject comprising concurrently,

a) treating an area of the skin of the subject, wherein treating comprises applying means for enhancing penetration and/or barrier disruption of the skin; and

b) applying a formulation transcutaneously to the treated area to induce an antigen-specific immune response, wherein the formulation comprises:

1) an antigen in an amount effective to induce an antigen-specific immune response;

2) an adjuvant present in an amount effective to enhance the immune response to the antigen; and,

3) a pharmaceutically acceptable carrier;  
wherein treating enhances the immune response.

Claim 294 (new): The method of claim 293, wherein treating comprises applying to the skin a chemical means, a physical means, a mechanical means, a hydration means, or a combination thereof.

Claim 295 (new): The method of claim 293, wherein treating comprises applying a chemical to the skin.

Claim 296 (new): The method of claim 295, wherein the chemical is an alcohol, an acetone, a detergent, a depilatory agent, a keratinolytic formulation, or a combination thereof.

Claim 297 (new): The method of claim 293, wherein treating comprises applying a device.

Claim 298 (new): The method of claim 297, wherein the device is selected from the group consisting of a propellant device, a device comprising tines, a device comprising microneedles, a device comprising a tine disk, a tape stripping device, a gas powered gun, a swab, an emery board, an abrasive pad, an electroporation device, an ultrasound device, and an iontophoresis device.

Claim 299 (new): The method of claim 297, wherein a patch comprises the device.

Claim 300 (new): The method of claim 293, wherein the antigen is a nucleic acid, carbohydrate, a glycolipid, a glycoprotein, a lipid, a lipoprotein, phospholipid, a polypeptide, a protein, a fusion protein, or chemical conjugate of a combination thereof.

Claim 301 (new): The method of claim 293, wherein the antigen is derived from a pathogen.

Claim 302 (new): The method of claim 301, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

Claim 303 (new): The method of claim 302, wherein the virus is an influenza virus or a rabies virus.

Claim 304 (new): The method of claim 303, wherein the antigen is hemagglutinin A.

Claim 305 (new): The method of claim 302, wherein the bacterium is *E. coli* or *Bacillus anthracis*.

Claim 306 (new): The method of claim 305, wherein the antigen is *E. coli* heat-labile enterotoxin (LT).

Claim 307 (new): The method of claim 305, wherein the antigen is a nucleic acid encoding *E. coli* heat-labile enterotoxin (LT).

Claim 308 (new): The method of claim 291, wherein the antigen is a pathogen.

Claim 309 (new): The method of claim 308, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

Claim 310 (new): The method of claim 309, wherein the virus is a whole virus, a live virus, an attenuated live virus, an inactivated virus, a detergent treated virus, or a combination thereof.

Claim 311 (new): The method of claim 310, wherein the virus is an influenza virus or a rabies virus.

Claim 312 (new): The method of claim 311, wherein the influenza virus comprises hemagglutinin A.

Claim 313 (new): The method of claim 309, wherein the bacterium is *E. coli* or *Bacillus anthracis*.

Claim 314 (new): The method of claim 313, wherein the *E. coli* comprises *E. coli* heat-labile enterotoxin (LT).

Claim 315 (new): The method of claim 291, wherein the antigen is a multivalent antigen.

Claim 316 (new): The method of claim 291, wherein the adjuvant comprises a molecule selected from the group consisting of a bacterial ADP-ribosylating exotoxin (bARE), a binding B subunit of a bARE, a toxoid of a bARE, a genetically altered bARE, and a genetically detoxified mutant of a bARE.

Claim 317 (new): The method of claim 316, wherein the antigen is an influenza antigen.

Claim 318 (new): The method of claim 291, wherein the adjuvant is a nucleic acid encoding a molecule selected from the group consisting of a bacterial ADP-ribosylating exotoxin (bARE), a binding B subunit of a bARE, a toxoid of a bARE, a genetically altered bARE, and a genetically detoxified mutant of a bARE.

Claim 319 (new): The method of claim 318, wherein the nucleic acid encodes *E. coli* heat labile enterotoxin (LT).

Claim 320 (new): The method of claim 293, wherein the antigen and the adjuvant are the same molecule.

Claim 321 (new): The method of claim 320, wherein the molecule is *E. coli* heat-labile enterotoxin (LT).

Claim 322 (new): The method of claim 320, wherein the molecule is hemagglutinin A.

Claim 323 (new): The method of 293, wherein the formulation is applied using a patch.

Claim 324 (new): The method of claim 293, wherein the adjuvant is selected from the group consisting of nucleic acid, bacterial exotoxin, cytokine, chemokine, lipopolysaccharide, a molecule containing unmethylated CpG motifs, a heat shock protein, a derivative of a heat shock



protein, tumor necrosis factor, genetically detoxified toxin, and combinations thereof.

Claim 325 (new): The method of claim 293, wherein the adjuvant is provided as a nucleic acid comprising a sequence encoding the adjuvant.

Claim 326 (new): The method of claim 293, wherein the antigen or adjuvant activates an antigen presenting cell.

Claim 327 (new): The method of claim 326, wherein the antigen presenting cell is a Langerhans cell or a dermal dendritic cell.

Claim 328 (new): The method of claim 293, wherein the antigen is a whole microorganism, a whole cell, or a virion.

Claim 329 (new): A method for inducing an antigen-specific immune response in a subject comprising:

- a) delivering parenterally a first formulation comprising an antigen to a subject;
- b) treating an area of the skin of the subject, wherein treating comprises applying means for enhancing penetration and/or barrier disruption of the skin to enhance the immune response; and
- c) applying transcutaneously a second formulation comprising an adjuvant to the area of the skin, thereby inducing an antigen-specific immune response.

Claim 330 (new): The method of claim 329, wherein treating comprises applying to the skin a chemical means, a physical means, a mechanical means, a hydration means, or a combination thereof.

Claim 331 (new): The method of claim 329, wherein treating comprises applying a chemical to the area of the skin.

Claim 332 (new): The method of claim 331, wherein the chemical is an acetone, a detergent, a depilatory agent, a keratinolytic formulation, or a combination thereof.

Claim 333 (new): The method of claim 329, wherein treating comprises applying a device.

Claim 334 (new): The method of claim 333, wherein the device is selected from the group consisting of a propellant device, a device comprising tines, a device comprising microneedles, a device comprising a tine disk, a tape stripping device, a gas powered gun, a swab, an emery board, an abrasive pad, an electroporation device, an ultrasound device, and an iontophoresis device.

Claim 335 (new): The method of claim 333, wherein a patch comprises the device.

Claim 336 (new): The method of claim 329, wherein the antigen is a nucleic acid, a carbohydrate, a glycolipid, a glycoprotein, a lipid, a lipoprotein, phospholipid, a polypeptide, a protein, a fusion protein, or chemical conjugate of a combination thereof.

Claim 337 (new): The method of claim 329, wherein the antigen is derived from a pathogen.

Claim 338 (new): The method of claim 337, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

Claim 339 (new): The method of claim 338, wherein the virus is an influenza virus or a rabies virus.

Claim 340 (new): The method of claim 339, wherein the antigen is hemagglutinin A.

Claim 341 (new): The method of claim 338, wherein the bacterium is *E. coli* or *Bacillus*

*anthracis*.

Claim 342 (new): The method of claim 341, wherein the antigen is *E. coli* heat-labile enterotoxin (LT).

Claim 343 (new): The method of claim 340, wherein the antigen is a nucleic acid encoding *E. coli* heat-labile enterotoxin (LT).

Claim 344 (new): The method of claim 329, wherein the antigen is a pathogen.

Claim 345 (new): The method of claim 344, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

Claim 346 (new): The method of claim 345, wherein the virus is a whole virus, a live virus, an attenuated live virus, an inactivated virus, a detergent treated virus, or a combination thereof.

Claim 347 (new): The method of claim 346, wherein the virus is an influenza virus or a rabies virus.

Claim 348 (new): The method of claim 347, wherein the influenza virus comprises hemagglutinin A.

Claim 349 (new): The method of claim 345, wherein the bacterium is *E. coli* or *Bacillus anthracis*.

Claim 350 (new): The method of claim 349, wherein the *E. coli* comprises *E. coli* heat-labile enterotoxin (LT).

Claim 351 (new): The method of claim 329, wherein the antigen is a multivalent antigen.

Claim 352 (new): The method of claim 329, wherein the adjuvant comprises a molecule selected from the group consisting of a bacterial ADP-ribosylating exotoxin (bARE), a binding B subunit of a bARE, a toxoid of a bARE, a genetically altered bARE, and a genetically detoxified mutant of a bARE.

Claim 353 (new): The method of claim 352, wherein the antigen is an influenza antigen.

Claim 354 (new): The method of claim 329, wherein the adjuvant is a nucleic acid encoding a molecule selected from the group consisting of a bacterial ADP-ribosylating exotoxin (bARE), a binding B subunit of a bARE, a toxoid of a bARE, a genetically altered bARE, and a genetically detoxified mutant of a bARE.

Claim 355 (new): The method of claim 353, wherein the nucleic acid encodes *E. coli* heat labile enterotoxin (LT).

Claim 356 (new): The method of claim 329, wherein the adjuvant is selected from the group consisting of nucleic acid, bacterial exotoxin, cytokine, chemokine, lipopolysaccharide, a molecule containing unmethylated CpG motifs, a heat shock protein, a derivative of a heat shock protein, tumor necrosis factor, genetically detoxified toxin, and combinations thereof.

Claim 357 (new): The method of claim 329, wherein the adjuvant is provided as a nucleic acid comprising a sequence encoding the adjuvant.

Claim 358 (new): The method of claim 329, wherein the antigen or adjuvant activates an antigen presenting cell.

Claim 359 (new): The method of claim 358, wherein the antigen presenting cell is a Langerhans cell or a dermal dendritic cell.

Claim 360 (new): The method of claim 329, wherein the antigen is a whole microorganism, a whole cell, or a virion.

Claim 361 (new): The method of claim 329, wherein the first formulation is administered subcutaneously, intradermally, or intramuscularly.

Claim 362 (new): The method of claim 329, wherein the second formulation is applied using a patch.